GROWTH AND AMINO ACID CONTENT OF FOOD YEAST ON DIFFERENT NUTRIENT MEDIA

BY

NUTULAPATY UMAMAHESWARA RAO

B.S., Benares Hindu University, India, 1946
M.S., University of Illinois, 1949

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Introduction

With the discovery of water soluble vitamins in recent years, considerable attention was focussed on yeasts as sources of these vitamins. Although it has been known for many years, that yeast is rich in protein, it was used as a supplement to the diet mainly because of the corrective value of the vitamins it contains. While an enormous amount of information is available on yeast from this standpoint, it is only recently that the public interest has been directed to the fact that yeast might prove of great economic importance for its protein content, particularly during emergencies.

The importance of amino acids in diet has assumed more significance in recent years, because of the researches of Rose, Holt, Murlin, Block, and others. The essential amino acids for human nutrition have been determined and the values for tyrosine, tryptophan, cystine, histidine, and arginine in animal, plant, and yeast proteins have been reported. According to the German workers (Aries 1946) very little is known as to the amounts of other essential amino acids, methionine, threonine, leucine, and phenylalanine in yeasts. This investigation was concerned with the effect of growing yeasts on various media, on yields, protein content, and the contents of the amino acids, methionine, threonine, leucine, and phenylalanine.

Historical

During the 19th and 20th centuries the science of Microbiology centered around the pathogenic forms of microscopic life. The work which led to the appreciation of the nutritional value of yeasts, had its roots (Thaysen and Morris 1943) in an economic problem, the disposal of surplus quantities of yeast from the brewing plants of Europe and U.S.A. every

year. By the end of 19th century, chemical analysis established that brewers' yeast contained a high percentage of protein. Delbrück (1914) was one of the first to recognise its value as food. With the end of war 1914-1918, and particularly after the publication of Osborne and Mendel (1919) who showed that rats could thrive well on a diet containing brewers' yeast as the sole source of protein, great interest had been shown in the nutritional value of yeasts for their protein content. It was found (Macrae et al 1942) that a comparatively small addition of yeast protein, to a diet containing only cereal protein of low dietetic value as maize protein, greatly increased the value of the latter in the growth of pigs. Yeast protein is being used in cattle feeding (Ritzman 1946) and in human nutrition (Thaysen and Morris 1943).

Experimental

The yeasts *Torula utilis No. 41, Saccharomyces cerevisiae No. 139, Rhodotorula gracilis No. 1091,* and *Candida guilliermondia* were obtained from professor F. M. Clark, Department of Bacteriology, University of Illinois. In experiments involving 24 hours incubation only the first three yeasts were used. Candida guilliermondia was also used in experiments where the cells were harvested after 72 hours' growth.

Since wood sugar is being successfully used in Germany for the commercial production of food yeast, and blackstrap molasses in Jamaica, West Indies, for the same purpose, both were employed in the present investigation, as carbohydrate sources. The wood sugars were obtained from Dr. E. E. Harris, Forest Products Laboratory, Madison 5, Wisconsin. The two wood sugars were 120A, a low pentose sugar with 85% hexoses and 15% pentoses and 120B, a high pentose sugar, containing 65% hexoses and 35% pentoses. The blackstrap molasses used were from three different sources: Urbana, Illinois, U.S.A.; The K.C.P.Ltd, Vuyyuru, Madras state, India; and The Bodhan Sugar Factory, Hyderabad, India. All the above three molasses contained approximately 50% hexoses.

Seventeen different nutrient media were employed to study the effects of their constituents on the yield, protein content, and the constitution of the cell proteins of the yeasts studied. Seven of these seventeen media were employed in the 72 hours growth experiments and fourteen of them in the 24 hours growth experiments. The wood sugars and the samples of blackstrap molasses were added in amounts such that twenty grams of hexoses were added per liter medium.

Nielsen and Rojowski (1950) reported that the protein content of *Rhodotorula gracilis* would vary between 12.0 & 50.0 per cent depending upon the amount of sulfur present in the nutrient medium. Hence ammonium chloride and ammonium sulfate were used as nitrogen sources in this study in the various media employed. Besides these, vitamin-free casamino acids were used as a nitrogen source, to test whether there would be any influence on the constitution of the cell proteins, due to the possible intact assimilation of these amino acids. As a fourth nitrogen source asparagine was used.

Since homocystine has often been suggested as a possible precursor in the synthesis of methionine, it was added to some of the media to test its influence on the methionine content of the cell protein. To some media magnesium sulfate was added as a supplement to determine its effects on protein and amino acid content.

The final pH of the media prepared was 5.5. For the various tests 500 milliliters of each medium were placed into a 3.0 liter Erlenmeyer flask, and sterilized for 20 minutes at 15 pounds pressure per square inch. All incubations were on a shaker at 25°-28°C.

For the estimations of the four essential amino acids, methionine, leucine, threonine, and phenylalanine, the method of Barton-Wright (1946) was employed with slight modifications. The test organism for the assays of the amino acids was *Leuconostoc mesenteroides P60*. It was obtained from the Department of Dairy Bacteriology, University of Illinois.

Summary

Microbiological assays were described for the estimation of methionine, threonone, leucine, and phenylalanine in the yeast cell hydrolyzates.

From the standpoint of yields blackstrap molasses, either from India or the United States, were found to be definitely superior to wood sugars, as substrates for the production of food yeasts.

Ammonium chloride, ammonium sulfate, and vitamin-free casamino acids were found to be equal in value, as nitrogen sources for the growth of yeasts on molasses used in this work. Only slight variations in protein content, and amino acids, methionine, threonine, leucine, and phenylalanine were obtained using these different nitrogen sources. The significant increase in yields of yeasts, when asparagine was substituted for ammonium sulfate at the 0.5 percent level, in the blackstrap molasses media, was not accompanied by an increase in the percentage of cell protein.

With yeasts grown on media, used in both the 24 and 72 hours growth experiments, the increase in yields after 72 hours incubation was not accompanied by a similar increase in the percentage of cell protein.

In general, the percentage of cell protein as well as the concentration of the four essential amino acids; methionine, threonine, leucine, and phenylalanine seem to be independent of the nutrient media used in this work, when *Torula utilis No. 41, Saccharomyces cerevisiae No. 139, Rhodotorula gracilis No. 1091,* and *Candida guilliermondia* were used as test organisms.

Rhodotorula gracilis No. 1091 had the highest concentration of methionine, among the three yeasts used in the 24 hour growth experiments. *Saccharomyces cerevisiae No. 139* had the next highest concentration, while *Torula utilis No. 41* contained the lowest amounts.

Torula utilis No. 41 and *Saccharomyces cerevisiae No. 139*

appear to be closely related and have slightly higher contents of threonine than *Rhodotorula gracilis No.1091.*

The amounts of leucine in all the three species were almost the same. In the case of phenylalanine, the values for all the above three species were closely related, except in one instance where *Torula utilis No. 41* grown for 24 hours, on a medium containing Urbana molasses and ammonium chloride, had a very low phenylalanine content.

In the 72 hours growth experiments, the concentrations of methionine in all the four organisms were on the same level. However, *Torula utilis No. 41* and *Saccharomyces cerevisiae No. 139* have higher contents of threonine, leucine, and phenylalanine than *Rhodotorula gracilis No. 1091* and *Candida guilliermondia.* The concentrations of phenylalanine were considerably lower in *Torula utilis No. 41, Rhodotorula gracilis No. 1091,* and *Candida guilliermondia* when they were grown on media containing asparagine.

Considering the yields, percentage protein, and the contents of the four amino acids analysed, *Torula utilis No. 41* was the best, and *Candida guilliermondia* was the poorest. *Rhodotorula gracilis No. 1091* was about equal to *Torula utilis No. 41* and better than *Saccharomyces cerevisiae No. 139.*

It was suggested that *Rhodotorula gracilis No. 1091* could be used as a food yeast on a commercial scale, because of its high yields and methionine contents when grown on various media, and the presence of carotenoid pigments.

REFERENCES

Aries, R.S., 1946. Northeastern Wood Utilization Council Bulletin. No. 12:76.

Barton-Wright, E.C., 1946. Nature 157:628.

Macrae, J.F., et al. 1942. Biochem. J. 35:460.

Nielsen, N. and Rojowski, P., 1950. Acta. Chemica. Scand. 4:1390.

Osborne, T.B. and Mendel, L.B., 1919. J. Biol. Chem. 38:223.

Ritzman, E.G., 1946. Northeastern Wood Utilization Council Bull. No. 12:9.

Thysen, A.C. and Morris, M., 1943. Nature 152:526.

VITA

The author was born on October 15, 1924., at Perukalapudi, Madras state, India. He graduated from G. C. B. High School, Thurumella, in 1941. He received his first two years of college education at Andhra Christian College, Guntur, and received his B.Sc. degree in 1946 from Benares Hindu University, Benares, India. From 1946 to 1948, he worked as an assistant chemist in the K. C. P. Ltd., a sugar factory at Vuyyuru, Madras state, India.

The author came to the United States of America in February 1948 to join the Graduate College, University of Illinois and took his M.S. degree in Bacteriology, in 1949. He was a research assistant in the Department of Bacteriology during 1949-1950 and 1951-1952.

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